

at daily and weekly interval using freshly prepared standard disc for comparison.

(5) *Assay*—(i) *Individual discs*. On each of three plates prepared as directed in paragraph (b)(3) of this section, place standard disc and two or more discs from each batch to be tested. The standard disc and the sample discs are placed on the plates in a circular pattern with random arrangement, with no disc being closer than 24 millimeters (on centers) to any other disc. Discs are placed on the plates within as short a period of time as possible (not to exceed 3 minutes per plate) and tapped gently to ensure an even seal. After incubation as directed in paragraph (b)(3) of this section, measure the diameter of each circle of inhibition as accurately as possible. (In most cases, it is possible to estimate diameters to the nearest 0.1 millimeter).

(ii) *Estimation of potency*. Determine the logarithm of each dose of standard (x values) and the mean zone diameter for each dose of standard (y values). Using the three values of x and the three corresponding values of y, calculate Σx , Σx^2 , $(\Sigma x)^2$, Σy , and Σxy . Calculate the regression coefficient (slope, b) and the Y-intercept (a) of the standard response line by using the following equations:

$$b = \frac{n\Sigma xy - (\Sigma x)(\Sigma y)}{n\Sigma x^2 - (\Sigma x)^2}$$

$$a = \frac{\Sigma y - b\Sigma x}{n}$$

where n = the number of standard doses.

Determine the zone diameter (Y) for each sample disc being tested. Using the regression equation

$$X = \text{antilog} \frac{Y - a}{b}$$

calculate the concentration (X) for the mean response (Y) of the sample discs.

(6) *Potency*. The potency of the batch is satisfactory if the mean result obtained for the batch is not less than 85 percent and not more than 150 percent of that represented.

[45 FR 20668, Apr. 6, 1979]

§ 460.15 Streptomycin sulfate discs for use in culture media.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Streptomycin sulfate discs for use in culture media are paper discs intended for impregnation of culture media in the sensitivity testing of mycobacteria. They conform to all requirements and to all procedures prescribed by § 460.1(a) for antibiotic sensitivity discs, except that each disc shall contain streptomycin sulfate equivalent to 10, 25, 50, or 500 micrograms of streptomycin.

(2) *Packaging*. It shall be packaged in accordance with the requirements of § 460.1(b).

(3) *Labeling*. In addition to complying with the requirements of § 460.1(c) of this chapter, the labeling shall also bear information indicating that the discs are for use in culture media for the sensitivity testing of mycobacteria and not for use in ordinary sensitivity disc plate tests.

(4) *Requests for certification; samples*. Requests for certification shall comply with § 460.1(d).

(b) *Tests and methods of assay; potency*. Proceed as directed in § 460.6 for the assay of streptomycin sulfate discs, except that:

(1) In the assay of streptomycin sulfate discs labeled to contain the equivalent of 10, 25, or 50 micrograms of streptomycin, the control discs shall be made to contain the equivalent of 6.25, 12.5, 25, 50, and 100 micrograms of streptomycin per disc.

(2) In the assay of streptomycin sulfate discs labeled to contain the equivalent of 500 micrograms of streptomycin:

(i) To each 100 milliliters of seed agar used for the test add 2.0 milliliters of suspension number 11.

(ii) The control discs shall be made to contain the equivalent of 50, 100, 200, 400, and 800 micrograms of streptomycin per disc.

§ 460.16 Rifampin discs for use in culture media.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Rifampin discs for use in culture media are paper discs intended for impregnation of culture media in